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SYNOPSIS

1. INTRODUCTION

Biologically significant molecules and pharmaceuticals in different matrices can be investigated electroanalytically by voltammetric method. Electrochemical measurements offer specificity, selectivity, high sensitivity and low detection limit depending on the modification of the electrode substrate. Therefore, research and development for the analyses of pharmaceuticals and biologically significant molecules must adopt improvised and innovative procedures in order to achieve accuracy, precision, selectivity, sensitivity and cost effectiveness. It was observed that different electrochemical techniques using different modified electrodes have displayed remarkable electrical potential making the system effective for the detection of biologically significant molecules and pharmaceuticals [1].

Carbon-based electrodes such as carbon paste electrode (CPE), glassy carbon electrodes (GCE) carbon fiber paper (CFP), etc. have received great attention as they are advantageous in terms of renewability, stable response, low ohmic resistance and non-requirement of internal solutions [2]. CFP offers abundant reduction and oxidation reaction sites due to high porosity and wettability. On modification, the conductivity and electroactive surface area tends to increase significantly [3]. Therefore, we have chosen CFP as the working substrate electrode. Chemical modifiers like conducting polymers can be introduced on to the surface of the electrode by electrochemical methods in order to achieve lower detection limits. These polymers comprise of carbon, hydrogen and heteroatoms (oxygen, nitrogen, sulfur) and contain π-conjugation across the polymer backbone. Conducting polymers (CPs) such as polyacetylene (PA), polyaniline (PANI), polyparaphenylene (PPh), polypyrrole (PPy), polythiophene (PTh) and poly(3,4-ethylenedioxythiophene) (PEDOT)

provide good conductivity and are most sought after supporting catalyst materials. The inherent conductivity of these CPs results from the formation of polarons (radical cations) and bipolarons. In electrochemical polymerization, the radical-radical coupling leads to the formation of a radical dication. This loses two protons to form a neutral dimer, which can be oxidised to a radical cation and reacts further to form the polymer [4]. Perhaps an adherent polymer film can be grafted directly onto an electrode surface in one simple step. Reproducible films can be obtained as the film thickness can be controlled by monitoring the charge passed during deposition and the polymer films formed have fewer impurities as harsh oxidising agents are not used. Improved electrical conductivity, mechanical strength and adhesive property to the substrate are important advantages offered by CPs thereby making them very useful in various applications. Electrodeposition of conducting polymers on a substrate electrode induces porosity and roughness due to their three-dimensional growth on the substrate, which enhances the surface area of CP substrate [5]. This favours electrode-electrolyte interfacial properties for various electrochemical reactions, wherein CPs transfer electrons between electrode and dispersed metal nanoparticles along the chains of polymer. CPs also prevent the agglomeration of nanoparticles. Therefore, electrooxidation or electroreduction efficiency can be improved by introducing metal nanoparticles into the porous conducting matrices, which in turn increases the surface area [6].

The activity of noble metal based electrocatalysts depends on their dispersion. CPs are useful for supporting catalyst particles. Different metal particles can be directly deposited on the polymer by direct chemical or electrochemical reactions between CP and metal cations. Electrochemical formation of mono/bi-metallic electrocatalysts on CP layer has been reported for the electrocatalytic oxidation of formic acid, methanol, other organic molecules and sensing of some organic and inorganic analytes [7].

Metal nanoparticles dispersed on electrically conducting polymers have gained momentum due to their various applications as sensors for biological systems, supercapacitors, corrosion protective coatings and electrocatalysts. Improved chemical stability, lower redox potential values, superior electronic conductivity and ease of formation makes PEDOT and PPy, efficient conducting host matrices for electrocatalytic applications. Only limited works on the dispersion of noble metal nanoparticles on PEDOT or PPy thin films have been reported for sensing applications [6]. Also, the aggregation of nanoparticles due to high surface energy could be overcome by employing carbonaceous support materials like CFP as they are good matrices in terms of high specific surface area and electric conductivity for the immobilization of CPs and nanoparticles dispersed in CPs [8]. CFP is chemically inert and possess outstanding mechanical and electrical properties that makes it an excellent base electrode for electrophysiological, electrochemical and biosensor applications on a micrometer and a nanometer scale.

Another emerging area of research among carbon-based materials for electrode modification is the synthesis of activated mesoporous carbon nanospheres (CNS) from natural sources. Earlier, CNSs were synthesized from graphite powder, coal, petroleum products, polymers and liquid/gaseous hydrocarbons, but these precursors have become scarce and impact the environment negatively. Therefore, the vital need to explore carbon materials from environment friendly and cost-effective carbon precursors, led to utilizing natural resources for the production of CNS. The natural resources such as plant biomasses, agriculture bio-wastes and hydrocarbons are ample, massive and diversified and can act as renewable carbon precursors. Remarkably, CNS have till date been synthesized by using carbon precursors (e.g., hemicelluloses and lignocelluloses) present in some of the natural resources such as rice husks, mango leaves, banana stem, banana fibers, apricot shell, corn grain, coconut shell, sago bark and

bamboo. The natural lignocellulosic components have gained considerable attention due to their availability, low cost and low toxicity. Therefore, CNS derived from natural resources have been utilized for a number of applications. CNS possess distinctive advantages such as regular surface morphology, high porosity, high chemical stability, uniform particle size, high specific surface area, good dispersion properties, low toxicity and excellent biocompatibility when compared to other forms of carbon, like activated carbon, carbon nanotubes and graphene. Generally, carbon-based functional nanomaterials are synthesized by chemical vapour deposition (CVD), nanocasting methods, electric arc discharge technique, carbonization of sugars, polymeric aerogels and MOFs [9]. The requirement of harsh and energy demanding synthetic conditions makes these methods disadvantageous. Therefore, we have employed an environmentally benign, clean, catalyst free and cost-effective process for the production of CNS using onion peel.

2. REVIEW OF LITERATURE

Among various analytical methods reported for the determination of analytes, electrochemical techniques have gained momentum due to their ease of operation, rapid response, low limit of detection and high sensitivity. Electrochemical sensors possess advantages like ease of fabrication, high electroactive surface area, voltammetric performance and enhanced electrochemical activity [10]. Surface modification of electrodes by immobilizing recognition elements on the sensor substrates is a very efficient approach to reach enhanced current responses and obtain an interface with highly specific binding affinity to the desired molecule [1]. Based on literature survey, biologically significant molecules such as morin, cholesterol, progesterone, methylmalonic acid and an anti-hypertensive pharmaceutical, Olmesartan medoxomil (OM) have been chosen as the analytes of interest.

The electrochemical determination of the flavonoid, morin has gained considerable importance over conventional analytical methods because of cost effectiveness and distinct approaches towards the method development, which enhances sensitivity and selectivity towards the analyte. Literature survey reveals that very few reports exist for the use of modified electrodes in the electrochemical determination of the flavonoid morin. When metal nanoparticles such as Pt, Pd, Ru, Au, etc. are uniformly dispersed and immobilized on a supporting host matrix, a thin film of conducting polymer (CP) display stronger adsorbability, greater catalytic activity and roughening of conductive sensing interface [11]. Some of the reports available in the literature are dispersion of Pd nanofibers on PANI films, Pd nanoparticles dispersed on PPy functionalized graphene, dispersion of Pt particles on PANI, PPy and poly-(2-hydroxy-3-aminophenazine) for electrocatalytic oxidation of various analytes [12]. Ir was found to be highly stable and exhibits high metallic conductivity when compared to Ru. Irshad and Munichandraiah have already demonstrated the electrocatalytic efficiency of Ir in electrochemical reactions. Therefore, to the best of our knowledge, the high electrocatalytic efficiency of Ir towards oxidation of organic molecules are yet to be explored [13].

Most of the reports for OM determination are based on HPLC methods. Electroanalytical methods offer advantages over other analytical methods in terms of simplicity, cost, rapid detection, requirement of sample in small quantity, non-requirement of derivatizations and less sensitivity towards matrix effects. A work on electrodeposition of Pt on PANI modified electrode based on cyclic voltammetry has described high electrocatalysis and long term stability for the oxidation of toluene [14]. Only few works on the dispersion of Pt particles on PEDOT thin film has been reported [6]. Also, electrochemical sensing performance can be enhanced by considering the synergistic effects of binary composition interface notably the bimetallic composites. Pt-based bimetallic systems

with different nanoparticles have been efficient and novel structures with significantly enhanced electrocatalytic activities in methanol oxidation and oxygen reduction reactions in fuel cell applications. Variations in surface-morphology, composition, catalytic activity and chemical selectivity of the material were reported on addition of second metal when compared to the single metallic nanocatalyst. PtRu bimetallic nanocatalysts supported on 3D-graphene foam was prepared and characterized by C. C. Kung and his coworkers and used in the electrochemical biosensing of H_2O_2 [15]. Only few reports on the use of first row transition metals and second or third row transition elements like Ru and Ir are available in the literature for designing bimetallic electrocatalytic systems. Ir was highly stable and displayed greater metallic conductivity when compared to Ru. Few reports have suggested superior electrocatalytic behaviour and stability of Ir in electrochemical reactions [13]. From the literature, it can be inferred that the bimetallic nanocatalyst particles dispersed on a conducting polymer displays greater catalytic activity than same materials in the absence of conducting polymers. To the best of authors' knowledge, no study has reported the catalytic behaviour of OM by electrochemical method based on electrodeposition of bimetallic PtIr nanostructures on PEDOT modified carbon fiber paper (CFP) substrate.

In the recent years, electrochemical biosensing have gained momentum over various analytical methodologies due to their rapid detection, reproducibility and selectivity with wide linear dynamic range. A number of works based on polyaniline/graphene, nanographite, graphene/β-cyclodextrin, nickel oxide/graphene, platinum nanoparticles/carbon nanotubes, stainless steel micro-needle/nanoporous platinum, silver nanotubes and molecularly imprinted polymers have been reported for the determination of Cholesterol. Usually, cholesterol esterase (ChEt) and cholesterol oxidase (ChOx) enzymes are used in the fabrication of Cholesterol biosensors. In these types of enzymatic reactions,

concentrations of either H_2O_2 generated or oxygen consumed during the process is monitored. The shortcomings of these methods include denaturation of enzymes due to sensitivity towards temperature and pH, reagent instability and high cost of the enzymes. Moreover, enzymatic biosensors tend to lose their catalytic property and structural integrity on repeated usage [16]. They exhibit poor response especially at high concentrations of Cholesterol when all the binding sites of enzymes get saturated. This is particularly significant when the system may have an extended range of detection. Researchers have used various hybrid systems to increase the electron transfer wherein the redox nanoparticles are dispersed on conducting polymer coated substrates that can facilitate the conversion of an irreversible oxidation process into a reversible one [17]. The electrochemical deposition of amorphous metal-based catalysts from buffer solutions has been used for oxygen evolution reactions (OER) [13]. Literature reports indicate that electrodeposition of metal ions such as Co^{2+}, Mn^{2+}, Fe^{2+}, Ni^{2+} and Cu^{2+} in phosphate, acetate or borate buffer solutions on substrate electrodes contained substantial amounts of anions from the buffer medium. Surprisingly, no reports exist in the literature on the use of these materials in the field of electrochemical sensing. The synergistic effects of electrochemical dispersion of Ru-based catalyst from phosphate buffer solution on conducting polymer modified carbon substrate is not been explored yet. In the present investigation, Ru-phosphate (Ru-Pi) was electrochemically deposited from phosphate buffer solution (pH 7.0) on PPy modified carbon fiber paper substrate.

Human metabolism as well as energy production requires small amounts of methylmalonic acid (MMA) which is a dicarboxylic acid and C-methylated derivative of malonate. If sufficient amount of Vitamin B_{12} is not available, then the concentration of MMA begins to increase which cause high levels of MMA in blood as well as in urine. Hence MMA is a specific metabolic marker and more sensitive indicator of functional

Vitamin B_{12}. The accepted values of MMA in healthy humans are between 73-271 nmol/L. MMA concentration in blood can be measured by techniques such as calorimetry based on the ion-exchange chromatography followed by diazotization with p-nitroaniline, gas chromatography of dimethyl ester of MMA obtained after the treatment with diazomethane, electrophoresis and high-pressure liquid chromatography (HPLC) coupled to tandem mass spectrometry or liquid chromatography-mass spectrometry. But, no electrochemical method has been reported till date for the determination of MMA in spite of its superior advantages. One of the major problems that are faced while sensing the biomolecules is that they tend to undergo oxidation at the closed potentials of conventional electrodes. In addition to this, the electrodes have fouling effect which causes poor selectivity and reproducibility. To overcome such problems modification of electrodes play a vital role in effective detection of biomolecules [18].

Distinctively, electrochemical techniques have received great attention in recent years as a number of modified electrodes have been reported for the quantification of hormones. There are reports which focused on the electrochemical determination of Progesterone using non-immunosensing based approaches [19]. However, quantification of Progesterone is challenging as its concentration is very low in biological matrices. Therefore, nature of the electrode modifier plays a vital role in designing reliable electrochemical sensors. As miniaturization of microelectronic devices is in great demand, sensors and biosensors based on different nanomaterials as modifiers have replaced organic redox mediators. Carbon-based materials at the nanoscale have exhibited advantages over conventional electrodes owing to their high electrical conductivity and enhanced surface area. Carbon-based materials possessing chemical stability and affinity to absorb organic or inorganic molecules. CNSs are highly porous and possess an open-pore network of carbon matrix that

facilitate rapid molecular diffusion and promote accessibility to the entire pore surface. The presence of surface functional groups on natural resources derived CNS exhibit excellent electrocatalytic activity for the determination of various species in different sources and are known to reduce the operating potential [20]. However, CNSs are not explored to the fullest for electrocatalytic applications even though its physical and chemical functions are similar to CNTs and graphene.

Voltammetric sensors based on modified electrodes were used for the analysis of a wide range of materials in the recent years as it increases the quality of electrochemical analysis. Based on the extensive literature survey done for electrochemical sensing of biomolecules and pharmaceuticals, the following research gaps have been identified:

- Need for developing facile electrode fabrication methods
- Demand for electrochemical sensors with very low detection limit values
- Non-availability of methods with high reproducibility and specificity
- Metal nanoparticles dispersed in conducting polymer matrices are majorly reported for oxidation of alcohols and seldom reported as electrochemical sensors
- High electrocatalytic efficiency of metals like Ir, bimetallic PtIr, amorphous Ru-Pi, bimetallic PdAu and CNS towards oxidation of organic molecules are yet to be explored extensively
- Need for application of the methods in real sample analysis

Therefore, research involved in the development of sensors is becoming one of the most sought-after scientific areas to achieve efficiency and overall benefit for the detection and quantitation of analytes of interest.

The aim of this study is to develop electrochemical sensors by chemical modification of electrodes for the detection of pharmaceuticals and biologically significant molecules. The proposed works involve various electrochemical studies involving metal nanoparticles dispersed in

conducting polymers such as PEDOT and PPy which finds its scope in various applications like electrocatalysis, sensors, electrochromic devices, supercapacitors, fuel cells, batteries and photovoltaic devices. Moreover, determinations of biomolecules and pharmaceuticals are thrust area in the future works related to fabrication of electrochemical sensors based on metal nanoparticles immobilised CP coated electrodes as it gives insights into the broad area of electrocatalysis.

3. OBJECTIVES

- To modify electrode materials for electrochemical sensors
- To characterize the modified electrode materials by different analytical techniques
- To optimize the experimental conditions necessary for the electroanalytical studies of the analytes
- To fabricate electrochemical sensors for the detection of pharmaceuticals and biologically significant molecules

4. RESEARCH WORK CARRIED OUT

4.1 General Methodology

Following are the steps involved in the proposed research works carried out.

- ✓ Fabrication of electrochemical sensors by modifying carbon fiber paper substrate electrodes using metal nanoparticles dispersed on conducting polymers by electrochemical methods.
- ✓ Characterization of modified electrodes by SEM (Scanning Electron Microscopy), TEM (Transmission Electron Microscopy), XPS (X-ray photoelectron spectroscopy), XRD (X-ray Diffraction), Raman spectroscopy, FT-IR spectroscopy (Fourier Transform Infrared) analyses.
- ✓ Electrochemical studies were carried out by using cyclic voltammetric, differential pulse voltammetric and electrochemical impedance spectroscopy techniques. Optimization of experimental conditions.
- ✓ Developed methods were used for the analysis of real samples containing pharmaceuticals and biologically significant molecules.

4.2 Work 1 - Electrocatalytic Oxidation of Morin on Electrodeposited Ir-PEDOT Nanograins

Morin is a naturally occurring flavonoid rich in anti-oxidant properties. Sasikumar et al. synthesized vanadium pentoxide nanoflakes (V_2O_5 NF) using ionic liquid and employed V_2O_5 NF film modified GCE for the determination of morin in fruit samples. This method was found to be less sensitive and selective when compared to the proposed method [21]. Varghese, Chitravathi, & Munichandraiah have effectively utilized the hydrogen bonding property offered by the polymer host matrix [electropolymerized dimercapto-1,3,4-thiadiazole [p-(DMTD)] modified carbon fiber paper electrode for the determination of morin (Figure 1). They have also demonstrated its application in the determination of morin in mulberry leaves. But the method could not illustrate electron transfer kinetics due to low conductivity of the polymer [22]. In the present work, nanoclusters of Ir were electrochemically deposited on carbon fiber paper (CFP) substrate modified with poly(3,4-ethylenedioxythiophene) (PEDOT), a conducting polymer. Initially, PEDOT was electrochemically deposited on CFP electrode using an electrolyte containing EDOT (0.01 M), SDS (0.01 M) and H_2SO_4 (0.1 M). 40 successive cycles were employed between -0.1 and +1.6 V vs SCE at a scan rate of 0.05 V/s for deposition of PEDOT on CFP electrode. PEDOT coated CFP electrode was rinsed several times with H_2SO_4 (0.1 M) solution, followed by drying. SDS has been used as a surfactant in order to improve the adherence between the substrate and PEDOT. Ir-PEDOT/CFP electrode was prepared by depositing Ir in 0.002 M $IrCl_3$ + 0.1 M H_2SO_4 voltammetrically in the range 0.0 - 1.6 V vs SCE at a scan rate of 0.05 V/s (25 cycles). Ir was deposited on PEDOT modified CFP and on bare CFP electrodes for comparative studies. For the electrochemical determination of morin by cyclic voltammetry, a glass cell containing 20 mL PBS (pH 7.0) and morin solution (15×10^{-10} M) was used between the potential 0.0 V and 0.6 V at 0.05 V/s scan rate. The

quantitative determination of morin was carried out by DPV technique in the potential range 0.0 – 0.6 V with 0.05 V (pulse amplitude), pulse width of 0.06 s and pulse period of 0.5 s. The anodic peak currents of the analyte were measured at 1.90 V vs SCE and a calibration graph was constructed for quantitative analysis of morin. The electrocatalytic activity of Ir–PEDOT/CFP electrode towards oxidation of morin was significantly greater than that of PEDOT/CFP and bare CFP electrodes. Factors affecting the anodic peak of morin namely, effect of pH, scan rate and number of cycles were optimized. Under optimal conditions, the linear dynamic range for the determination of morin was found to be 0.12 nM to 2.80 nM (Figure 2). The significantly low detection limit (42.18 pM) demonstrates the ultrasensitivity of the proposed method. The reliability of the method was evaluated for the quantification of morin present in mulberry leaves, guava leaves and grape wine. The proposed work has been schematically represented in Figure 3. Similar methodologies were followed for experiments carried out in Work 2, Work 3, Work 4 and Work 5 and have been briefed in this synopsis.

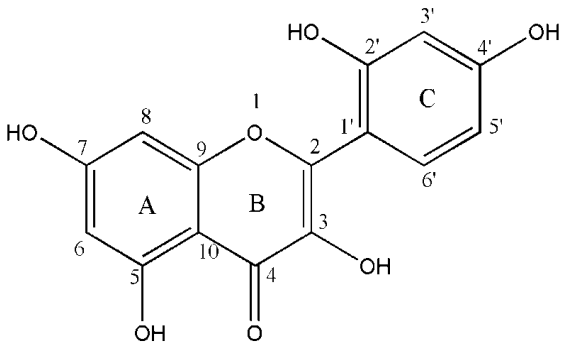

Figure 1. Structure of Morin.

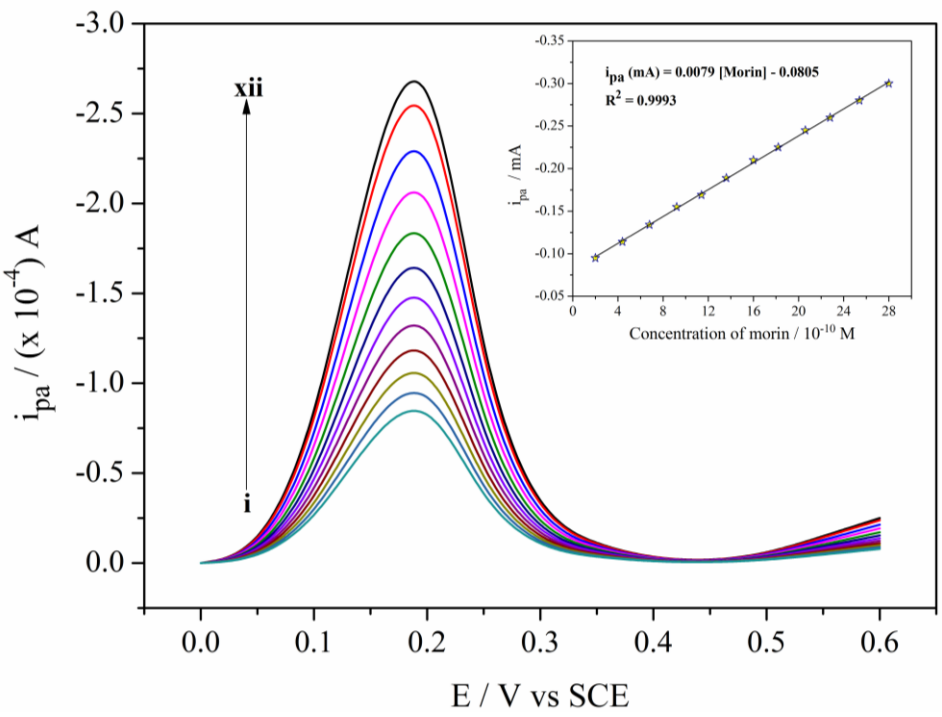

Figure 2. Differential Pulse Voltammograms of morin at different concentrations from (i) 0.12 nM to (xii) 2.80 nM in the potential range 0.0 V – 0.6 V vs SCE in PBS (pH 7.0). Inset is the calibration plot for determination of morin under same experimental conditions.

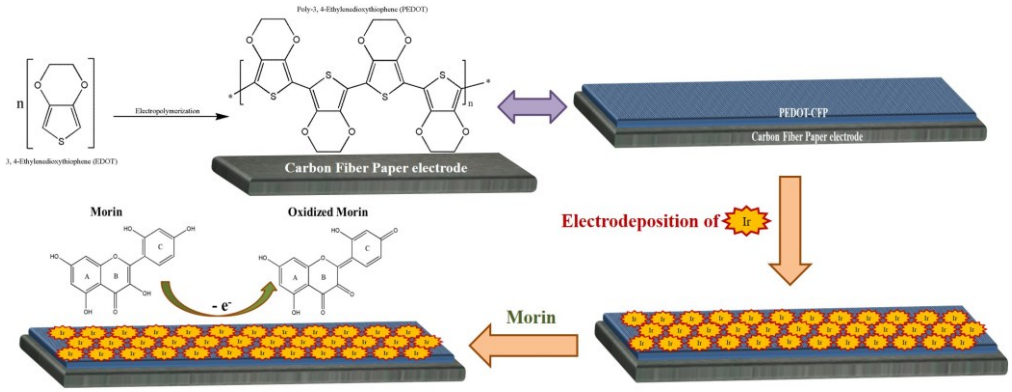

Figure 3. Graphical illustration for electrocatalytic oxidation of morin on electrodeposited Ir-PEDOT nanograins on carbon fiber paper electrode.

4.3 Work 2 - PEDOT Decorated with PtIr Nanoclusters on Carbon Fiber Paper toward Electrocatalytic Reduction of a Hypertensive Drug Olmesartan Medoxomil

Several analytical methods that have been reported for OM determination in biological fluids and pharmaceutical formulations are based on HPLC methods. Surprisingly, there are only three reports based on electroanalytical methods for OM determination in bulk and dosage forms. M. M. Rahman and his coworkers prepared low-dimensional nanoparticles composed of SnO_2-Co_3O_4 nanocubes (NC) on a silver electrode for the determination of OM in tablet dosage forms [23]. In another work reported by M. Celebier, OM was determined in pharmaceutical formulations using a hanging drop mercury electrode [24]. F. Ozturk and his coworkers investigated the electrochemical properties of OM at hanging mercury drop electrode. This was applied for direct determination of OM in pharmaceutical dosage form and human-urine without time-consuming steps prior to drug assay [25]. But, these methods suffer from disadvantages like high detection limits, narrow linear dynamic range and complicated electrode preparation procedures. Therefore, we have reported a facile electrochemical sensor which was developed by electrochemical deposition of PtIr nanoparticles on poly(3,4-ethylenedioxythiophene) (PEDOT) modified carbon fiber paper electrode (CFP). The modified electrodes were characterized by Field emission scanning electron microscopy (FESEM) with energy-dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), RAMAN spectroscopy, Fourier transform infrared spectroscopy (FTIR) and electroanalytical techniques. PtIr-PEDOT modified CFP electrode was used successfully in the determination of an anti-hypertensive prodrug Olmesartan medoxomil (OM) at femtomolar (fM) level for the first time (Figure 4). Under optimal conditions, the prepared electrochemical sensor has shown broad linear range between 0.001 pM and 0.220 pM and superior detection limit of 3.42

fM was observed based on Differential pulse voltammetric (DPV) study (Figure 5). In addition, the designed electrochemical sensor was successfully demonstrated as a novel platform in the electrochemical determination of OM in human urine and pharmaceutical samples.

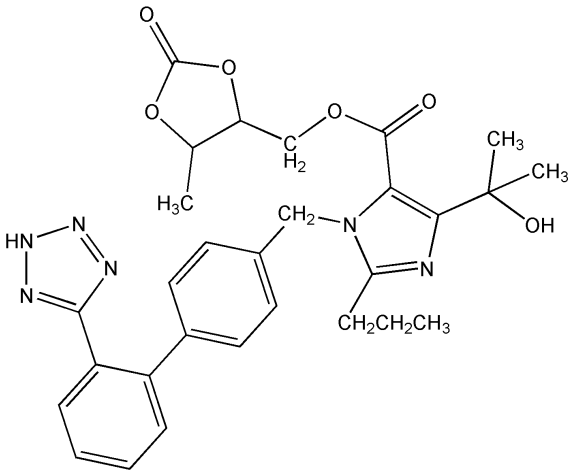

Figure 4. Structure of Olmesartan medoxomil.

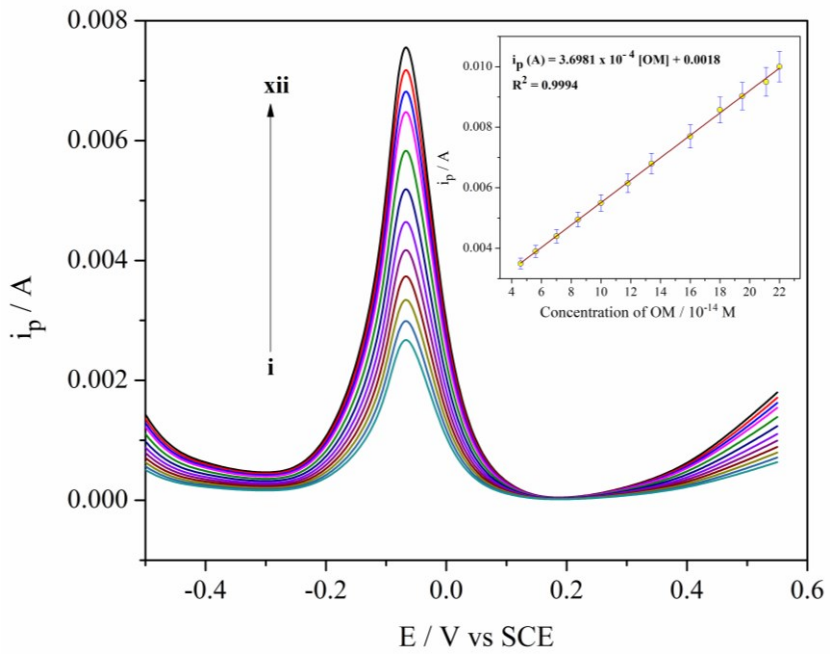

Figure 5. Differential Pulse Voltammograms of OM at different concentrations from (i) 0.001 pM to (xii) 0.220 pM. Inset is the calibration plot for determination of OM under same experimental conditions. Each error bar represents standard deviation for n = 3.

4.4 Work 3 - Amorphous Ru-Pi nanoclusters coated on polypyrrole modified carbon fiber paper for non-enzymatic electrochemical determination of Cholesterol

Literature review reveals that seldom reports exist for the non-enzymatic determination of Cholesterol [16], [26]. M. A. H. Nawaz and his coworkers fabricated an electrochemical sensor using a screen-printed carbon electrode (SPCE), multi-walled carbon nanotubes (MWCNTs) and β-cyclodextrin (β-CD). This developed sensor was applied in the non-enzymatic determination of cholesterol in human serum samples [27]. A novel Cu/Ni bimetal-dispersed carbon nanofiber/polymer nanocomposite (BMCP)-based electrode was developed by P. K. Bairagi and N. Verma. Dendritic nanofibers of poly methyl orange (PMO) grown on BMCP served as the recognition element for the analyte in the non-enzymatic electrochemical sensing of cholesterol [28]. In the present investigation, a facile electrochemical sensor based on Ruthenium-Phosphate (Ru-Pi) was developed by electrodeposition of Ru-Pi on Polypyrrole (PPy) modified carbon fiber paper (CFP) electrode. Phosphate buffer solutions of neutral pH containing $RuCl_3$ was used for voltammetric deposition of Ru-Pi on PPy/CFP electrode. The modified electrodes were physicochemically and electrochemically characterized. A significant amount of phosphate (Pi) and Ru centers in higher oxidation states were present on Ru-Pi film deposited on PPy/CFP substrate. Pi plays a significant role during catalyst deposition and in its activity towards determination of Cholesterol (Figure 6). DPV studies demonstrated that under optimum conditions, the developed sensor has displayed a wide linear dynamic range between 0.16 nM and 20.0 nM with a superior detection limit of 0.54×10^{-10} M (Figure 7). The proposed method was effectively applied in the non-enzymatic determination of Cholesterol at an ultralow level in human blood serum samples. The method displayed high selectivity towards Cholesterol in the presence of other interfering substances.

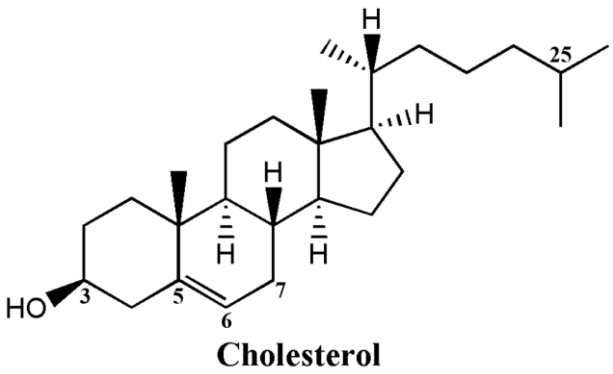

Figure 6. Structure of Cholesterol.

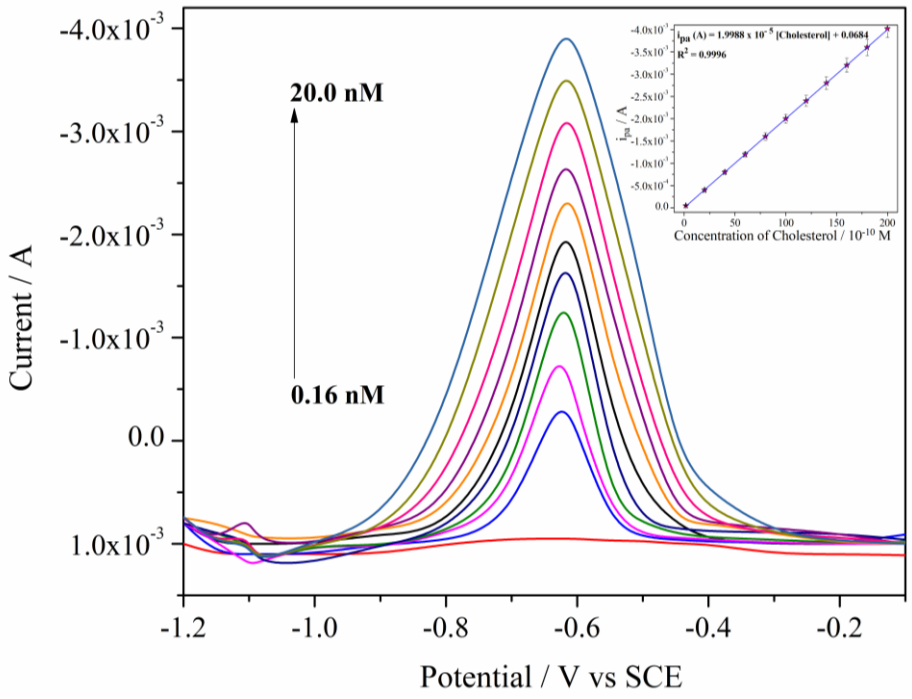

Figure 7. Differential Pulse Voltammograms of Cholesterol at concentrations from 0.16 nM to 20.0 nM. Inset is the linear calibration plot for quantitative determination of Cholesterol under similar experimental conditions.

4.5 Work 4 – PdAu-PPy tailored on carbon fiber paper electrode for the non-enzymatic electrochemical determination of methylmalonic acid

Vitamin B_{12} acts as a cofactor for two important enzymatic reactions. The conversion of Methyl malonyl CoA (a form of MMA) to Succinyl coenzyme A in mitochondria and for the conversion of homocysteine (HCY) to methionine. An excess of methylmalonyl CoA gets converted to MMA. Low levels of Vitamin B_{12} increase the levels of MMA in blood and urine. Clinical determination of MMA concentration in blood serum is by the method of gas chromatography, which is time consuming and not cost effective. An acceptable method for human Vitamin B_{12} in terms of accuracy, precision and rapidity is still unavailable. However, drawbacks such as inferior sensitivity and selectivity, costly set-up, cumbersome and time-consuming operation makes electrochemical method prevail above the conventional techniques [18]. Electrochemical sensing of MMA concentration in blood serum is possibly a reliable method for determination of Vitamin B_{12} status as electrochemical biosensors provide an effective strategy in analyzing the content of a biological sample due to the direct conversion of a biological content to an electronic signal. MMA (Figure 8) is very crucial for human metabolism as well as energy production. Determination of methylmalonic acid has received a great deal of attention in the determination of Vitamin B_{12} deficiency yet not a single electrochemical method has been reported. Herein, we report a facile electrochemical sensor based on Palladium Gold (PdAu) electrochemically deposited on polypyrrole (PPy) modified carbon fiber paper (CFP) electrode. The modified electrodes were characterized by analytical and electroanalytical techniques. Under optimal conditions, the prepared electrochemical sensor has shown broad linear dynamic range with a superior detection limit of 0.54×10^{-10} M based on Differential pulse

voltammetric (DPV) study (Figure 9). The proposed method was successfully used in the non-enzymatic determination of MMA at an ultralow level.

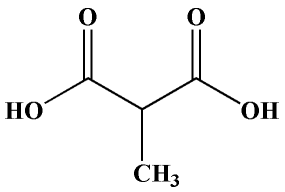

Figure 8. Structure of Methylmalonic acid.

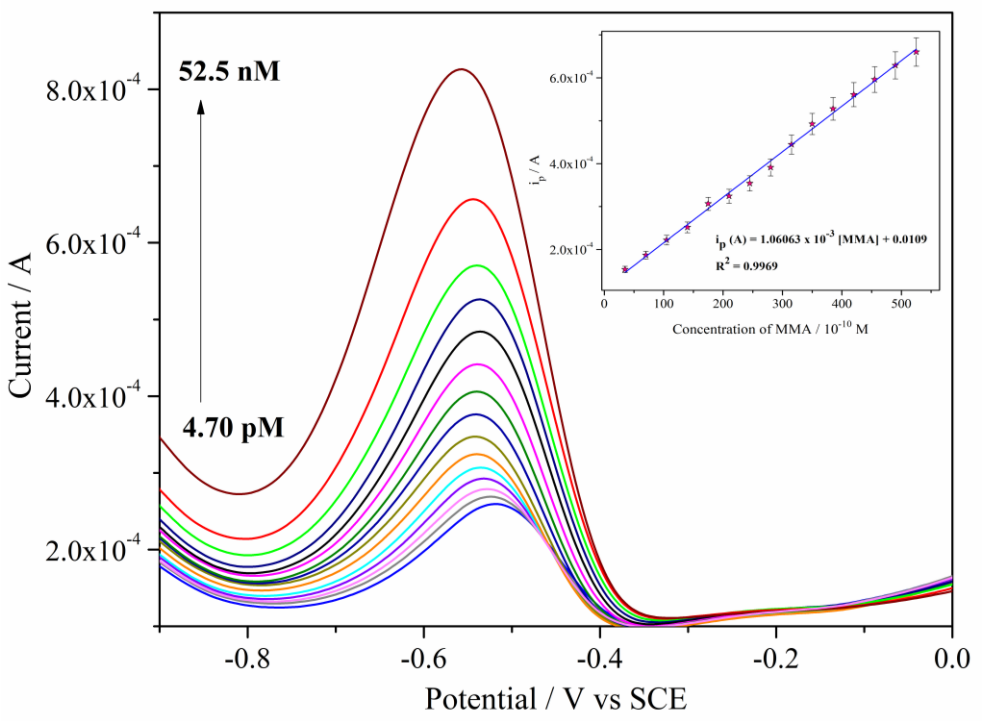

Figure 9. Differential Pulse Voltammograms of Methylmalonic acid at concentrations from 4.70 pM to 52.5 nM. Inset is the linear calibration plot for quantitative determination of Methylmalonic acid under similar experimental conditions. Each error bar represents standard deviation for n = 3.

4.6 Work 5 - Non-enzymatic electrochemical determination of progesterone using carbon nanospheres from onion peels coated on carbon fiber paper

A number of reports exist in the literature for the determination of progesterone based on molecularly imprinted and enzymatic approaches. But only few reports are available on the non-enzymatic determination of progesterone. M. Arvand and his coworkers modified glassy carbon electrode (GCE) with nanocomposites of PEDOT and ZrO_2 nanoparticles, which exhibited good conductivity and a three-dimensional-microporous network structure owing to the presence of nanoparticles and displayed excellent electrocatalytic activity towards the oxidation of progesterone in biological fluids and pharmaceutical products [29]. A. Gevaerd and her coworkers developed imidazole-functionalized graphene oxide modified GCE for the electrochemical determination of progesterone in pharmaceutical formulation [30]. M. Arvand and S. Hemmati fabricated a electrochemical sensor for progesterone based on graphene quantum dots (GQDs), Fe_3O_4 nanoparticles and functionalized multi-walled carbon nanotubes (f-MWCNTs) modified GCE [Fe_3O_4@GQD/f-MWCNTs/GCE]. The electrocatalytic properties of the modified electrode towards the oxidation of progesterone were analysed in human serum samples and pharmaceutical products [31]. In the present research work, we have utilized CNS derived from natural lignocellulosic components of onion peel to coat the CFP substrate. CNS were prepared from Onion peels utilizing an environmentally benign and cost-effective strategy. The CNS modified CFP electrodes were physicochemically characterized by Field emission scanning electron microscopy (FESEM) with energy-dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD) spectroscopy and X-ray photoelectron spectroscopy (XPS) techniques. Electrochemical characterizations of the modified electrodes were done by Cyclic voltammetry (CV) and Electrochemical impedance spectroscopy (EIS).

CNS modified CFP electrode was successfully used in the determination of Progesterone, an important steroid hormone (Figure 10) at an ultra-nanomolar level with superior detection limit of 0.012 nM and broad linear dynamic range of 37.39 pM – 0.25 nM (Figure 11). The developed electrochemical sensor was effectively utilized for the determination of Progesterone in pharmaceutical Progesterone injections, human blood serum samples and cow milk samples.

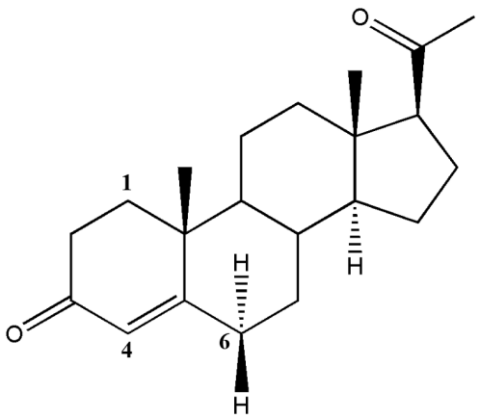

Figure 10. Structure of Progesterone.

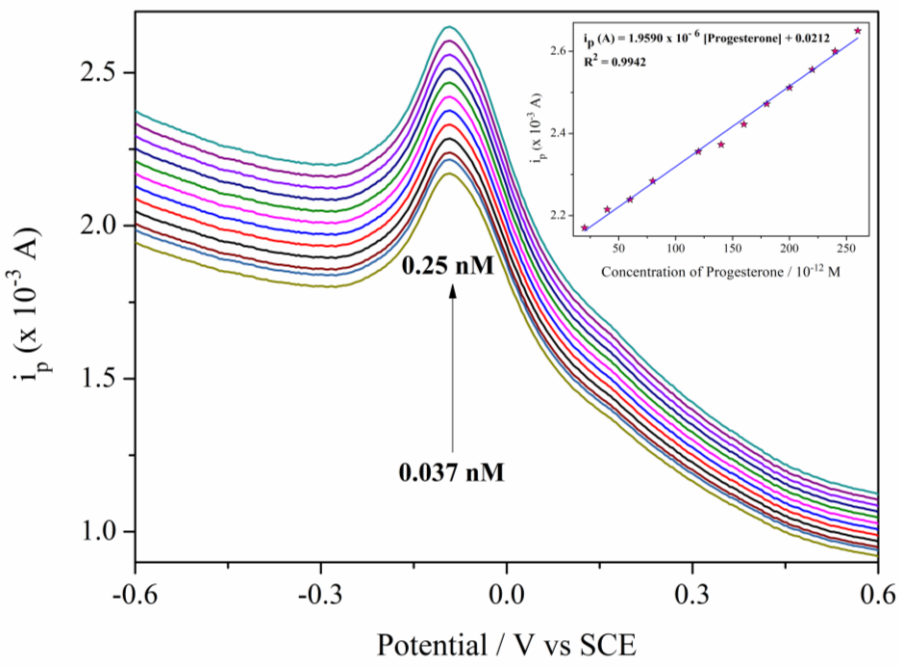

Figure 11. Differential Pulse Voltammograms of Progesterone at different concentrations from 0.037 nM to 0.25 nM. Inset is the calibration plot for determination of progesterone under same experimental conditions.

5 CONCLUSIONS

The electrochemical properties of Morin, Olmesartan medoxomil, Cholesterol, Methylmalonic acid and Progesterone at Ir-PEDOT/CFP, PtIr-PEDOT/CFP, RuPi-PPy/CFP, PdAu-PPy/CFP and CNS/CFP electrodes have been studied. These electrodes have displayed superior electrocatalytic activities towards oxidation/reduction of the listed analytes.

In the determination of morin, Ir-PEDOT/CFP electrode exhibited strong electrocatalytic activity. Morin oxidation was found to be adsorption-controlled, irreversible and a single-step process with the involvement of two electrons and two protons. Low detection limit value with high selectivity and sensitivity are the main advantages of the proposed method when compared to other reported methods. The developed method was effectively employed in the analysis of morin content present in mulberry leaves, guava leaves and grape wine.

The second research work reports a facile, highly sensitive and selective electrochemical method for the determination of Olmesartan medoxomil in pharmaceutical and biological samples. Highly dispersed PtIr nanoclusters in the PEDOT host matrix favours electroreduction of OM at large negative potential shift. This can be attributed to saturation of the azomethine group C=N in tetrazolyl moiety of OM and a femtomolar level detection limit was obtained which is first of its kind. The developed method was successfully applied to the assay of OM in its tablet formulations and human urine sample. Percentage of assay recovery shows that the method is free from interference of the excipients used in the formulations.

A simple, highly selective non-enzymatic electrochemical method has been reported for the quantitative determination of Cholesterol in human blood serum samples. The conducting polymer PPy possesses surface defects which behaves as nucleation centers for the growth of Ru-Pi

nanoclusters. The dispersed amorphous Ru-Pi nanoclusters in PPy host matrix favour the electrochemical oxidation of Cholesterol with a negative potential shift. This can be a result of oxidation of the -OH group in Cholesterol to –C=O group. A picomolar level detection limit was attained for the first time for a non-enzymatic determination of Cholesterol. The modified electrode could detect the analyte in the concentration from 0.16 nM to 20.0 nM with high selectivity. As well, a very low limit of detection (0.54×10^{-10} M) was achieved. The proposed method did not exert significant effects on interfering substances in the determination of Cholesterol.

A facile and sensitive non-enzymatic electrochemical sensor for the determination of MMA is developed. Bimetallic PdAu nanoparticles dispersed on PPy substrate facilitates electrocatalytic oxidation of MMA with a negative potential due to the oxidation of –OH group to =O group. An ultra-low level detection limit was obtained for the non-enzymatic determination of MMA. The proposed method was free from interferences and was effectively applied to determine MMA in human blood serum and urine samples.

A simple, highly sensitive and selective electrochemical sensor for the quantification of Progesterone in pharmaceutical injections, human blood serum samples and cow milk samples was reported in the fifth research work. The increased electroactive sites present on CNS coated CFP electrode due to the porosity of CNS facilitate electrooxidation of Progesterone at a negative potential, which can be attributed to oxidation of Progesterone at C-6 position which results in the formation of cationic radical intermediate which further undergoes oxidation in the presence of water resulting in the formation of oxidized Progesterone. An ultralow-level detection limit was obtained which is first of its kind. The developed method was found to be highly selective and free from interferences.

The thesis will be divided into seven chapters. Chapter 1 will be the introduction. Chapter 2 will be on the review of literature, which will cover the significant reported works for the electrode modification and electrochemical determination of chosen analytes. Chapter 3 describes electrocatalytic oxidation of Morin on electrodeposited Ir-PEDOT nanograins. Chapter 4 will brief on PEDOT decorated with PtIr nanoclusters on carbon fiber paper toward electrocatalytic reduction of a hypertensive drug Olmesartan medoxomil. Chapter 5 focuses on Amorphous Ru-Pi nanoclusters coated on polypyrrole modified carbon fiber paper for non-enzymatic electrochemical determination of Cholesterol. Chapter 6 will be based on dispersed PdAu nanoparticles on PPy coated carbon fiber paper for the electrochemical determination of MMA. Chapter 7 gives insights into Non-enzymatic electrochemical determination of Progesterone using carbon nanospheres from onion peels coated on carbon fiber paper. Chapter 8 briefs on the conclusions of the research work and this is followed by details on paper publications and conference proceedings.

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PUBLICATIONS AND PROCEEDINGS

I. Papers in Journals

[1] K. B. Akshaya, A. Varghese, Y. N. Sudhakar, and L. George, "Electrocatalytic oxidation of morin on electrodeposited Ir-PEDOT nanograins," *Food Chemistry*, vol. 270, pp. 78-85, 2019. (Impact Factor: **4.946**)

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II. Conference Proceedings

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